Award Number: W81XWH-14-1-0616

TITLE: Spinal Cord Injury-Induced Dysautonomia via Plasticity in Paravertebral Sympathetic Postganglionic

PRINCIPAL INVESTIGATOR: Shawn Hochman

CONTRACTING ORGANIZATION: Emory University
Atlanta, GA, 30322

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	30 Sep 2014 - 29 Sep 2015

4. TITLE AND SUBTITLE

Spinal Cord Injury-Induced Dysautonomia via Plasticity in Paravertebral Sympathetic Postganglionic

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0616

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S) **Shawn Hochman, PhD**

E-Mail:shochm2@emory.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Emory University
Atlanta, GA, 30322

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

ABSTRACT Sympathetic postganglionic neurons (**SPNs**) located in sympathetic ganglia represent the final common sympathetic motor output. Even though SCI produces a profound plasticity in sympathetic autonomic function, the extent that SCI-induced dysautonomia is based on SPN changes within the thoracic paravertebral sympathetic chain is unknown. Given their strategic site in autonomic signaling to body, any plasticity is likely to be of high significance, yet there is a paucity of studies undoubtedly due to their near anatomical inaccessibility. We have solved the accessibility problem with a strategic methodological advance. We will determine the extent to which paravertebral SPNs are a nodal site for vasomotor dysfunction after SCI.

We will undertake physiological, pharmacological and optogenetic studies to examine network and cellular plasticity induced by SCI to answer the following two questions: (a) Does SCI lead to plasticity in synaptic interactions between preganglionics, SPNs and primary afferents? (b) Do SPNs become hyperresponsive to synaptic inputs after SCI?

14.

15. SUBJECT TERMS

Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	*19b.* **TELEPHONE NUMBER** *(include area code)*

Contents

The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

1. INTRODUCTION:

Sympathetic postganglionic neurons (**SPNs**) located in sympathetic ganglia represent the final common sympathetic motor output. Even though SCI produces a profound plasticity in sympathetic autonomic function, the extent that SCI-induced dysautonomia is based on SPN changes within the thoracic paravertebral sympathetic chain is unknown. Given their strategic site in autonomic signaling to body, any plasticity is likely to be of high significance, yet there is a paucity of studies undoubtedly due to their near anatomical inaccessibility. We have solved the accessibility problem with a strategic methodological advance. We will determine the extent to which paravertebral SPNs are a nodal site for vasomotor dysfunction after SCI.

We will undertake physiological, pharmacological and optogenetic studies to examine network and cellular plasticity induced by SCI to answer the following two questions: (a) Does SCI lead to plasticity in synaptic interactions between preganglionics, SPNs and primary afferents? (b) Do SPNs become hyperresponsive to synaptic inputs after SCI?

2. KEYWORDS:

spinal cord injury, sympathetic, autonomic, autonomic dysreflexia, spinal cord, electrophysiology, plasticity, paravertebral, postganglionic

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

a. What were the major goals of the project?

1. *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.*

Characterizing thoracic chain sympathetic postganglionics		
Major Task 1a: Convergence and divergence	**months**	**% completion/ Completion dates**
Subtask 1: Segment specific properties	1-6	75%
Subtask 2: Pharmacology	7-12	*50%*
Subtask 3: Breeding/crossing transgenic mice and spinalizations	1-36	3months behind target
Subtask 3: Establish intracellular recording techniques	3-18	100%
Major Task 1b: Convergence and divergence	**months**	
Subtask 1: Incorporation of optogenetic approaches for selective activation of neuron populations	12-18	25%
Milestone(s) Achieved: Understanding of synaptic organization in uninjured mice and ability to use optogenetics to selectively activate afferent and efferent fiber populations		
Intracellular recordings and optogenetics		
Major Task 2: Characterize mechanisms responsible for dysautonomia after spinal cord injury using intracellular recordings and optogenetics	**months**	**% completion/ Completion dates**
Subtask 1: Physiological plasticity in preganglionic-postganglionic interactions assessed using optogenetics	18-36	0%
Subtask 2: Physiological plasticity in afferent-postganglionic interactions assessed using optogenetics	18-36	0%
Subtask 3: Physiological plasticity in preganglionic-afferent interactions assessed using optogenetics	18-36	0%
Subtask 4: Intracellular recordings of synaptic and cellular plasticity in membrane properties; demonstration of membrane bistability	18-36	5%
Milestone(s) Achieved: Demonstration of important contribution of thoracic sympathetic chain to SCI-induced autonomic plasticity and forward insight into therapeutic interventions for future study		
Data analysis and publications		
Major Task 3: Data analysis and publications	**months**	**% completion/ Completion dates**
Subtask 1: Data analysis	6-36	25%
Subtask 2: Manuscript writing and submission	24-36	10%
Milestone(s) Achieved: Dissemination of scientific results.		

b. What was accomplished under these goals?

1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative);

Convergence and divergence of inputs in thoracic chain ganglia. **[Fig 1]** Properties of preganglionic divergence and convergence onto thoracic chain SPNs was previously examined in guinea pig [6,7]. We began to undertake similar studies in the adult mouse and observed comparable patterns of convergence and divergence. Moreover recruited of presynaptic events covered a comparable range of conduction velocities [6].

Characterization of cellular properties in adult mouse thoracic paravertebral ganglia. **[Fig 2]** A major function of sympathetic paravertebral chain ganglia neurons is to maintain vasomotor tone. While the functional properties of cervical and lumbosacral paravertebral ganglia neurons have been characterized, little is known about the functional properties of neurons within thoracic paravertebral ganglia. We developed an approach that allows for whole-cell patch clamp recordings in intact thoracic ganglia to characterize cellular and synaptic properties. We recorded from 8 cells deemed of good quality and obtained the following mean values ± SD: resting membrane potential (-66 ± 10 mV), membrane resistance (526 ± 235 MΩ), τ_m (54 ± 25 ms), and rheobase (76 ± 47 pA). Threshold voltage was typically 10 mV higher than resting membrane potential, action potentials displayed after-hyperpolarization and some cells displayed post-inhibitory rebound. All neurons were capable of repetitive firing. Maximal firing rates observed in response to depolarizing current steps ranged from 14-17 spikes/sec. During intracellular depolarization, firing rate increases with increased current injection and cells sustain tonic firing. Spike frequency adaptation was also observed. All recorded properties are fully consistent with those reported recently with whole cell recordings in rat superior cervical ganglia. Strikingly, our recorded properties differ substantially from sharp electrode recordings obtained from adult mouse tSPNs. Our recorded membrane resistance is 4.5 fold higher and τ_m is 7.5 fold longer than observed by Jobling and Gibbins (1999), and our neurons only fired tonically (e.g. Fig 4) while theirs only fired phasically to depolarizing current pulses. Note that all of these differences are consistent with greater cell damage caused by sharp electrode penetration compared to patch clamp recordings. We therefore assume that our new whole cell recordings are closer to physiological reality.

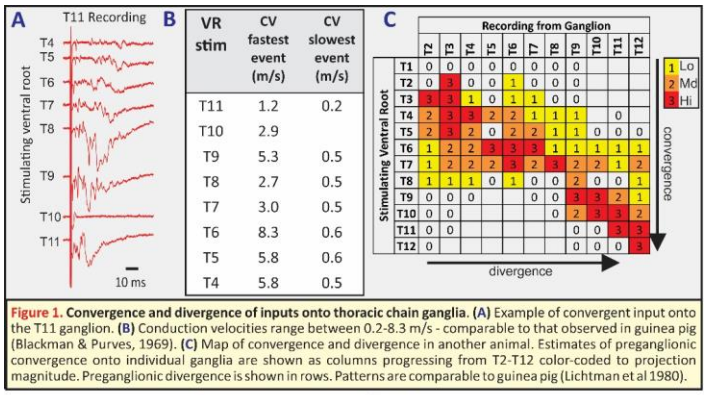


Figure 1. Convergence and divergence of inputs onto thoracic chain ganglia. **(A)** Example of convergent input onto the T11 ganglion. **(B)** Conduction velocities range between 0.2-8.3 m/s - comparable to that observed in guinea pig (Blackman & Purves, 1969). **(C)** Map of convergence and divergence in another animal. Estimates of preganglionic convergence onto individual ganglia are shown as columns progressing from T2-T12 color-coded to projection magnitude. Preganglionic divergence is shown in rows. Patterns are comparable to guinea pig (Lichtman et al 1980).

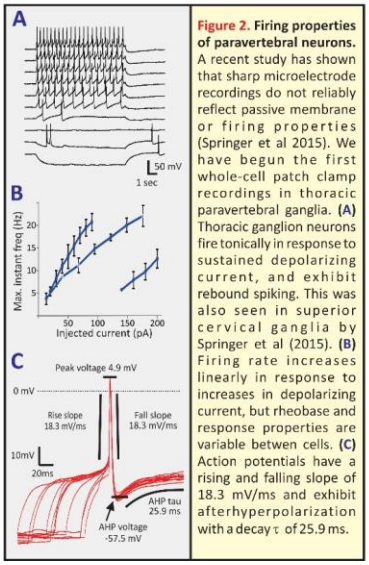


Figure 2. Firing properties of paravertebral neurons. A recent study has shown that sharp microelectrode recordings do not reliably reflect passive membrane or firing properties (Springer et al 2015). We have begun the first whole-cell patch clamp recordings in thoracic paravertebral ganglia. **(A)** Thoracic ganglion neurons fire tonically in response to sustained depolarizing current, and exhibit rebound spiking. This was also seen in superior cervical ganglia by Springer et al (2015). **(B)** Firing rate increases linearly in response to increases in depolarizing current, but rheobase and response properties are variable between cells. **(C)** Action potentials have a rising and falling slope of 18.3 mV/ms and exhibit afterhyperpolarization with a decay τ of 25.9 ms.

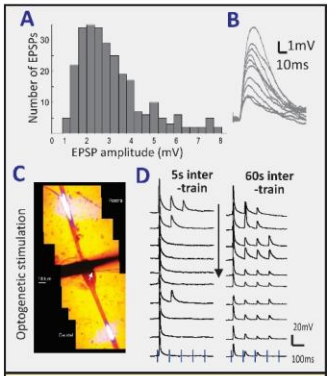


Figure 3. Synaptic properties of paravertebral neurons. **(A)** Spontaneous EPSP amplitudes occupy a continuous range from 1 to 8mV. **(B)** Overlaid traces of representative EPSPs. **(C)** We use optogenetics to selectively recruit preganglionic axons from the interganglionic nerve in ChAT::ChR2 mice. **(D)** Optogenetic recruitment of preganglionic axons led to large evoked EPSPs (20-40 mV) in the postganglionic cell. Responses fatigue rapidly with 5-pulse 10Hz trains when 5 sec inter-train intervals are provided and much less so at 60s intervals.

Patch clamp recordings in combination with optogenetics in another cell from a ChAT-ChR2 mouse suggested that postganglionic neurons receive massive cholinergic input from preganglionic neurons. Repetitive (10 Hz) optical stimulation of cholinergic preganglionic axons elicited a large EPSP in the postganglionic neuron (20 mV). The EPSP was usually suprathreshold and accompanied by an action potential. The response fatigued dramatically to repetitive stimuli. This seems to indicate that presynaptic release of acetylcholine attenuates rapidly on the order of 100-300 ms. Together, these data suggest that presynaptic release of acetylcholine by preganglionic neurons is the rate-limiting step for transmission in thoracic paravertebral ganglia.

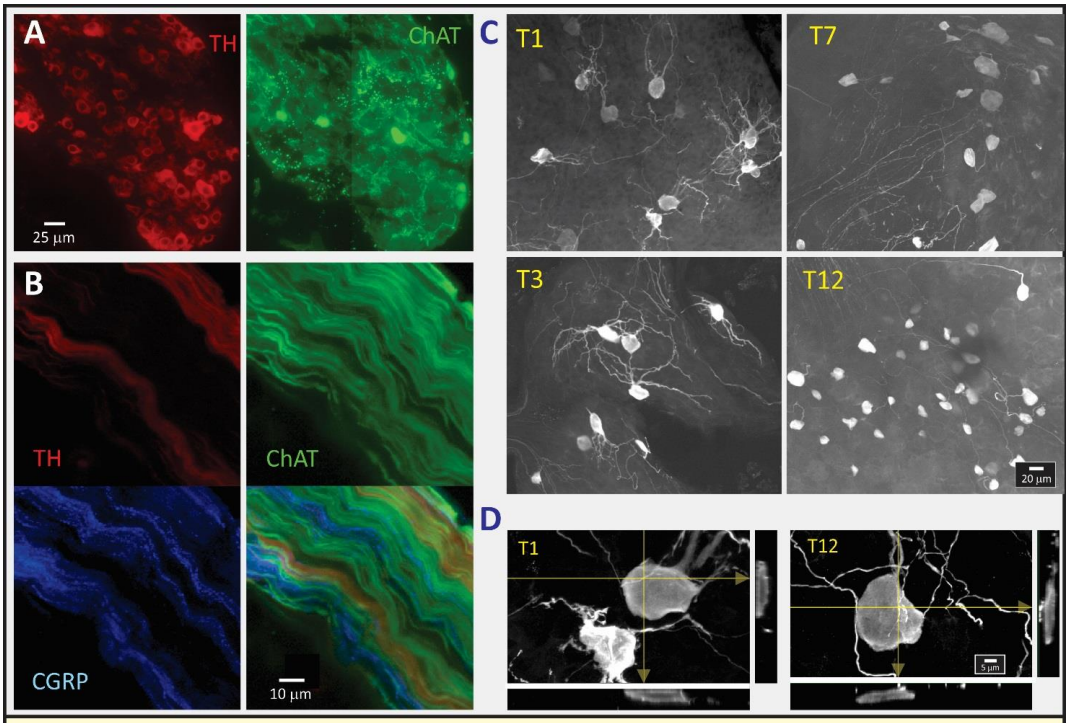


Figure 4. Neurochemistry and anatomy of thoracic chain ganglia. (A) Most cells are adrenergic and idenitified by immunolabeling for tyrsosine hydroxylase (TH), while a minority (3-5%) are cholinergic, identified by their expression of choline acetyltransferase (ChAT). **(B)** Axons within interganglionic nerves are TH+ postganglionic adrenergic, ChAT+ preganglionic, and CGRP+ visceral afferents. **(C)** Morphology of individual postganglionic neurons in several thoracic chain ganglia revealed by TH-Cre line with sparse labeling. While TH::tdTomato neurons from rostral thoracic chain ganglia contain dendrites (T1 and T3 are shown at left), more caudal thoracic ganglia lack clear dendritic arborizations (T7 and T12 are shown at right). Overall, TH neurons from T4-T12 ganglia did not contain observable dendrites. **(D)** TH::tdTomato labelled adrenergic neurons with putative somatic adrenergic synapses.

Synaptic properties of paravertebral neurons. **[Fig 3]** Spontaneous EPSP amplitudes occupy a continuous range. For the neuron shown, this was from 1-8mV. Using (**ChAT**)::channel rhodopsin (**ChR2**) mice, we optogenetically recruited cholinergic preganglionic synaptic properties in the interganglionic nerve and observed much larger amplitude EPSPs (20-40 mV) in the postganglionic cell. These EPSPs fatigued rapidly with inter-train periods of 5 sec, but not at 60 s intervals.

Anatomical properties of tSPNs. **[Fig 4]** Neurons within the sympathetic chain ganglia can be identified using TH and ChAT dependent reporter strains. Here, staining for GFP in a ChAT-GFP line shows the relative numbers of TH-immunolabeled adrenergic neurons to GFP-labeled cholinergic neurons (Fig 4A). Concomitant staining for CGRP positive primary afferents shows an interganglionic nerve composition with mutually exclusive adrenergic cholinergic CGRP$^+$ axons (Fig 4B).

We have begun to take advantage of a TH-Cre lines with notable sparse labeling[20]. Strikingly, we saw that, while TH$^+$ neurons in rostral thoracic ganglia contain dendrites, there is little evidence of dendrites in caudal thoracic chains (Fig 4C). The presence or absence of dendrites is an important factor in considerations of tSPN synaptic and firing properties in the modeling studies proposed. High magnification Confocal reconstructions suggest that adrenergic SPNs are synaptically interconnected (Fig 4D).

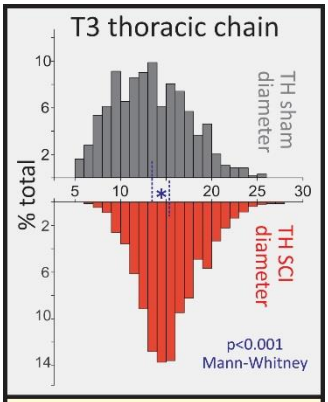


Figure 5. Mean SPN cell diameter increases after SCI. Distribution of TH$^+$ neurons in T3 ganglia of sham (gray) and 3 wk spinalized mice (red).

Plasticity after SCI. [Figures 5-8] Cell diameters of TH^+ neurons in the T3 thoracic chain ganglia were clearly increased in the after SCI (Fig 5). In recordings from the T12 ganglia from other SCI mice, 3 of 4 animals expressed persistent oscillatory activity that was between ~10-20Hz and long-lasting (seconds to over 30 minutes; Fig 6AB). Strikingly, spontaneous bursting events acted as switches between oscillatory and quiescent states, consistent with an induction of membrane bistability. Only 1/27 control mice exhibited this behavior (and only in atropine). 10 Hz ventral root stimulation evoked long-lasting bouts of increased activity in 2/4 SCI mice but never in control mice (0/27) (Fig 7). Induction of activity increases were dependent on activation preganglionic nAChRs (mecamylamine sensitive). How bistability induced in individual tSPNs can synchronize to induced network oscillations is uncertain. However, synaptic interactions between tSPNs is one possibility given observation of putative somatic adrenergic synaptic interconnections (Fig 8D). The consequence is reinforcement of adrenergic excitability by ongoing activity. For example applied norepinephrine leads to the emergence of population synchronous spontaneous spikes within the T12 ganglion that were of comparable magnitude to evoked responses following high threshold electrical stimulation of the T11 ventral root (Fig 9). Additional extra-synaptic autocrine and paracrine release of transmitters, possibly a prevalent feature of sympathetic ganglia[21] may also contribute to population synchronization.

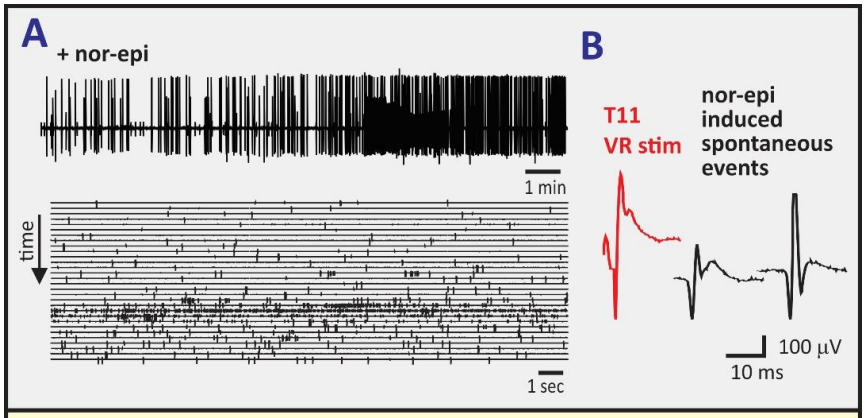


Figure 8. Adrenergic recruitment of spontaneous activity in T12 chain ganglion. **(A)** Top. Continuous recording following application of nor-epinephrine (100 µM). Bottom. Raster of same period showing 20 sec epochs of every 30 sec (period of evoked responses removed). **(B)** Ventral root stimulation-evoked response magnitude compared to emergent spontaneous events in the presence of nor-epinephrine.

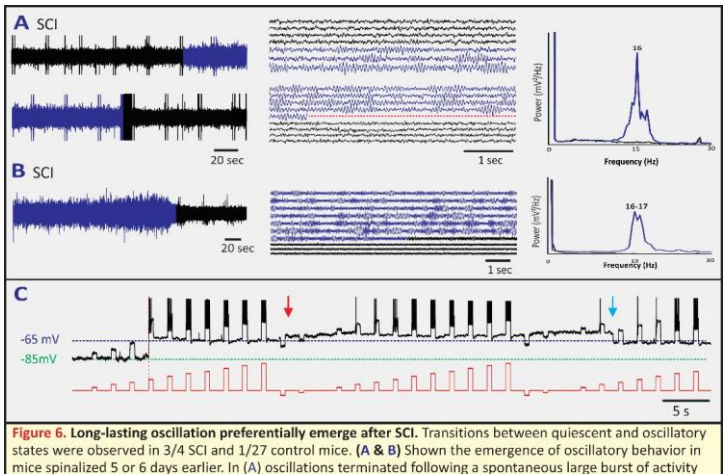


Figure 6. Long-lasting oscillation preferentially emerge after SCI. Transitions between quiescent and oscillatory states were observed in 3/4 SCI and 1/27 control mice. **(A & B)** Shown the emergence of oscillatory behavior in mice spinalized 5 or 6 days earlier. In (A) oscillations terminated following a spontaneous large burst of activity (bottom row). In (B) oscillations were induced following 10Hz stimulation. In both animals oscillations were at 16-17 Hz (right panels). **(C) Membrane bistability as possible cellular equivalent to population oscillatory responses.** T5 postganglionic neuron. Current ramps were delivered of different size and polarity as shown. A depolarizing pulse (at red vertical dotted line) led to a shift from a hyperpolarized (-85mV) to a more depolarized state (-65mV). Note also that the cell subsequently may have depolarized to a third excitable state on rebound from a hyperpolarizing pulse ~25s later before (red arrow), then spontaneously shifting back down to the first excitable state (cyan arrow). Spikes are shown truncated.

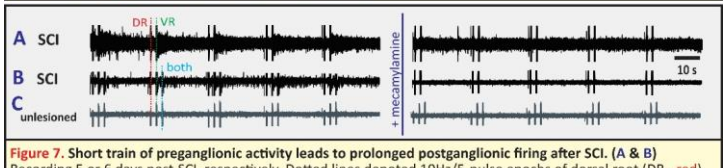


Figure 7. Short train of preganglionic activity leads to prolonged postganglionic firing after SCI. (A & B) Recording 5 or 6 days post-SCI, respectively. Dotted lines denoted 10Hz/5-pulse epochs of dorsal root (DR - red), ventral (VR - green) or co-stimulation (both - cyan). 10 Hz VR stimulation increased postganglionic firing that was abolished subsequent to application of the nAChR antagonist mecamylamine (50 µM; right panels). **(C)** Unlesioned mice never showed prolonged spiking after VR stim (0/27 mice). Trains were delivered every 30 sec.

4) other achievements.

- **Submitted a CRCNS grant application with a computational modeler as co-PI to try novel approaches that would hasten discovery while minimizing need for animals**
    - **Specific aims page is reproduced below**

SPECIFIC AIMS

Sympathetic postganglionic neurons (**SPNs**) represent the final common sympathetic motor output. Thoracic SPNs (**tSPNs**) located in paravertebral chain ganglia receive convergent input from preganglionic neurons, providing the dominant sympathetic control of vascular function in the trunk and upper extremities. Given their strategic nodal site in autonomic signaling to body, any plasticity in tSPNs is likely to be of high significance. Yet tSPNs are inaccessible for *in vivo* study, so operational principles are inferred from studies in cervical[1-3] and lumbar chain ganglia[4,5]. To date, only 3 *in vitro* studies have revealed tSPN electrophysiological properties[6-8], and there are still no accurate recordings of their cellular integrative properties or underlying recruitment principles.

We undertook THE FIRST PHYSIOLOGICAL STUDIES ON CAUDAL THORACIC CHAIN GANGLIA IN THE ADULT MOUSE by developing an *ex vivo* preparation with intact segmental preganglionic and rostrocaudal interganglionic connections[9,10]. We also obtained the FIRST WHOLE CELL RECORDINGS OF tSPN synaptic and cellular properties[11]. These data are a critical prerequisite to modeling studies, as observed synaptic integrative and firing properties are fundamentally different than previously observed with sharp electrodes due to impalement injury[3,8]. Our results already support tSPNs as overtly unique compared to SPNs characterized elsewhere. Here, we interleave experimental testing with modeling to understand tSPN recruitment principles and their integrative properties **[SA1]** as an essential launchpad to interrogate mechanisms that generate persistent bouts of hyperexcitability after spinal cord injury (**SCI**) **[SA2]**.

[SA1] Hypothesis. tSPNs have heterogeneous synaptic, cellular, and network properties, and are active participants in input-output recruitment strategies. We tuned parameters obtained from bullfrog SPNs [12,13] with our whole-cell recordings to generate a conductance-based computational model having linear f-I curve and spike frequency adaptation. We will generate population models of input-output relations starting with thoracic T5 and T12 ganglia. Both receive predominant preganglionic input from their segmental ventral roots and their axon numbers can be obtained from respective white rami. Combined with counts of total tSPNs in these ganglia, we will pair experiments and modeling conditions to study population recruitment principles and integrative properties sculpting tSPN output. *Experiments* will involve testing orderly recruitment of preganglionic axons (in ChAT::CHR2 mice)[14] by varying blue light intensity while recording individual and population tSPN responses to understand recruitment order of individual tSPNs relative to recruited population responses. *Modeling* will explore the rules of population recruitment relative to; (a) the fraction of preganglionic axons recruited, and (b) indexed over a range of frequency-dependent synaptic fatigue responses. tSPN population output will be compared when cellular properties are modeled as uniform [homogeneous] vs. having an experimentally determined synaptic input range and cell excitability [heterogeneous].

[SA2] Hypothesis. tSPNs convert from linear to non-linear gain amplifiers after SCI. Somatic output-encoding motoneurons are bistable and can alter output gain via recruitment of dendritic plateau potentials[15]. Strikingly, we observed that most tSPNs lack dendrites and rarely exhibit bistability. However, population oscillatory activity was triggerable after SCI[9] supporting emergent membrane bistability coincident with dendritic sprouting[16]. ①Experiments. Whole-cell recordings will compare differences in the response properties of tSPNs with (T2-3) and without dendrites (T5&12). Voltage and current clamp step and ramp protocols will test for activation of persistent inward currents (**PICs**) and expression of membrane bistability. Synaptically-evoked recruitment of tSPNs will determine whether bistability is synaptically-triggered while dye labeling will relate morphology to observed cellular responses. We will similarly compare caudal tSPN responses in control mice and after high thoracic SCI to assess whether bistability corresponds to dendritic sprouting, and also leads to persistent population voltage oscillations. ①Modeling with a two compartment neuron model that generates membrane bistability via insertion of a dendritic PIC will test the consequence of triggerable bistability to overall population output using an otherwise identical model to that in **SA1**. ②Modeling will generate hypotheses on factors contributing to autonomic dysreflexia (**AD**) - *a sudden, persistent sympathetic hyperactivity seen in people (and rodents) after high thoracic SCI* - by testing whether increases in synaptic strength (sprouting) and/or dendritic PICs (dendritic growth) alters tSPN cellular/population recruitment patterns consistent with AD. The model will also implement strategic parameter changes that simulate drug actions at known binding sites to test whether tSPNs are a plausible locus of AD therapeutics. ②Experiments will then use the *ex vivo* model to directly test for concordant drug-induced changes in tSPN excitability.

Overall significance. If successful, combined approaches will have finally uncovered the operational principles governing the final neural command pathways regulating vascular tone by generating an accurate and detailed electrophysiological database of thoracic chain ganglia. Aberrant increases in tSPN gain could lead to sympathetic hyperactivity in various autonomic disorders. SPN dysfunction may be pivotal to impaired function in cardiac arrhythmias, diabetes, oxidative stress, and essential hypertension[1,3,17-19]. A

database amenable to realistic modeling studies should be transformative to the field. Insights derived from probing the network parameter space for putative neural bases of emergent dysfunction, could catalyze novelty in both experimental testing and in drug discovery-based therapeutic considerations.

References:

1 Campanucci, V., Krishnaswamy, A. & Cooper, E. Diabetes depresses synaptic transmission in sympathetic ganglia by inactivating nAChRs through a conserved intracellular cysteine residue. *Neuron.* **66**, 827-834. doi: 810.1016/j.neuron.2010.1006.1010. (2010).

2 Rimmer, K. & Horn, J. P. Weak and straddling secondary nicotinic synapses can drive firing in rat sympathetic neurons and thereby contribute to ganglionic amplification. *Frontiers in neurology* **1**, 130, doi:10.3389/fneur.2010.00130 (2010).

3 Springer, M. G., Kullmann, P. H. & Horn, J. P. Virtual leak channels modulate firing dynamics and synaptic integration in rat sympathetic neurons: implications for ganglionic transmission in vivo. *The Journal of physiology* **593**, 803-823, doi:10.1113/jphysiol.2014.284125 (2015).

4 Bratton, B., Davies, P., Janig, W. & McAllen, R. Ganglionic transmission in a vasomotor pathway studied in vivo. *J Physiol.* **588**, 1647-1659. doi: 1610.1113/jphysiol.2009.185025. Epub 182010 Mar 185022. (2010).

5 Percy, W. H. & Krier, J. Cholinergic excitatory synaptic potentials of neurones in mammalian lumbar paravertebral ganglia. *Journal of the autonomic nervous system* **18**, 195-205 (1987).

6 Blackman, J. G. & Purves, R. D. Intracellular recordings from ganglia of the thoracic sympathetic chain of the guinea-pig. *J Physiol.* **203**, 173-198. (1969).

7 Lichtman, J. W., Purves, D. & Yip, J. W. Innervation of sympathetic neurones in the guinea-pig thoracic chain. *J Physiol.* **298**, 285-299. (1980).

8 Jobling, P. & Gibbins, I. L. Electrophysiological and morphological diversity of mouse sympathetic neurons. *Journal of neurophysiology* **82**, 2747-2764 (1999).

9 Halder, M. a. H., S. in *8th SFN Satellite Symposium on Motor Systems.*

10 Halder, M. S., M., and Hochman, S. . in *Soc. Neurosci. Abst. 40.*

11 McKinnon, M., Choi, M.H., and Hochman, S. . in *8th SFN Satellite Symposium on Motor Systems*

12 Karila, P. & Horn, J. P. Secondary nicotinic synapses on sympathetic B neurons and their putative role in ganglionic amplification of activity. *Journal of Neuroscience* **20**, 908-918 (2000).

13 Wheeler, D. W., Kullmann, P. H. & Horn, J. P. Estimating use-dependent synaptic gain in autonomic ganglia by computational simulation and dynamic-clamp analysis. *Journal of neurophysiology* **92**, 2659-2671, doi:10.1152/jn.00470.2004 (2004).

14 Llewellyn, M. E., Thompson, K. R., Deisseroth, K. & Delp, S. L. Orderly recruitment of motor units under optical control in vivo. *Nature medicine* **16**, 1161-1165, doi:10.1038/nm.2228 (2010).

15 Hultborn, H., Brownstone, R. B., Toth, T. I. & Gossard, J. P. Key mechanisms for setting the input-output gain across the motoneuron pool. *Progress in brain research* **143**, 77-95 (2004).

16 Lujan, H. L., Janbaih, H. & DiCarlo, S. E. Dynamic interaction between the heart and its sympathetic innervation following T5 spinal cord transection. *Journal of applied physiology (Bethesda, Md. : 1985)* **113**, 1332-1341, doi:10.1152/japplphysiol.00522.2012 (2012).

17 Krishnaswamy, A. & Cooper, E. Reactive oxygen species inactivate neuronal nicotinic acetylcholine receptors through a highly conserved cysteine near the intracellular mouth of the channel: implications for diseases that involve oxidative stress. *The Journal of physiology* **590**, 39-47, doi:10.1113/jphysiol.2011.214007 (2012).

18 Campanucci, V. A., Krishnaswamy, A. & Cooper, E. Mitochondrial reactive oxygen species inactivate neuronal nicotinic acetylcholine receptors and induce long-term depression of fast nicotinic synaptic transmission. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **28**, 1733-1744, doi:10.1523/jneurosci.5130-07.2008 (2008).

19 Shen, M. J. & Zipes, D. P. Role of the autonomic nervous system in modulating cardiac arrhythmias. *Circulation research* **114**, 1004-1021, doi:10.1161/CIRCRESAHA.113.302549 (2014).

Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

c. What opportunities for training and professional development has the project provided?

- *Two individuals were sent to Chicago this October to present their data as a poster in at a satellite symposium in conjunction with the Annual Society for Neuroscience Meeting.*

d. *Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives*.

- *Continue investigations as proposed with more focus on anatomical and cellular plasticity*

4. IMPACT:

Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

- **What was the impact on the development of the principal discipline(s) of the project?**
  - *Nothing to Report*
- **What was the impact on other disciplines?**
  - *Led to a CRCNS application with a computational neuroscientist*
- **What was the impact on technology transfer?**
  - *Nothing to Report*
- **What was the impact on society beyond science and technology?**
  - *Nothing to Report.*

5. CHANGES/PROBLEMS:

Nothing to Report

6. PRODUCTS:

Nothing to Report

Publications, conference papers, and presentations
Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1. M Halder and S Hochman. Early insight into emergence of spontaneous synchronous oscillatory activity in isolated thoracic chain ganglia after SCI. Pre-meeting on rhythmic motor circuits. 10/16/2015.
2. M McKinnon, M Choi, S Hochman. Characterization of cellular and synaptic properties in adult mouse thoracic paravertebral ganglia. Pre-meeting on rhythmic motor circuits. 10/16/2015.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

- *Hannah Choi – 5% effort – Honor's research Project*
- *Mallika Halder – 75% effort – research specialist*
- *Michal McKinnon – 90% effprt – graduate student*
- *Michael Sawchuk, - 75% effort - lab manager*

e. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- *Nothing to Report*

f. What other organizations were involved as partners?

- *Nothing to Report*

8. SPECIAL REPORTING REQUIREMENTS

9. APPENDICES:

g. abstracts,

1. **M Halder and S Hochman**. Early insight into emergence of spontaneous synchronous oscillatory activity in isolated thoracic chain ganglia after SCI. *Pre-meeting on rhythmic motor circuits. 10/16/2015.*

Department of Physiology, Emory University School of Medicine, Atlanta, GA
Paravertebral sympathetic postganglionic neurons (SPNs) located in thoracic chain sympathetic ganglia represent the predominant sympathetic control of vascular function in the upper and middle extremities. Loss of descending control of autonomic function as occurs after spinal cord injury (SCI) leads to a variety of autonomic changes that could arise in part from changes within paravertebral sympathetic chain SPNs. We developed an in vitro adult mouse model to examine evoked population responses in these ganglia following stimulation of segmental sympathetic preganglionic and primary afferents axons before and after spinal cord injury. The approach retains the adult mouse thoracic sympathetic chain ganglia in situ with connections to dorsal root ganglia, dorsal roots and ventral roots. Dorsal roots were stimulated to examine primary afferent evoked responses, and ventral root stimulation was used to recruit axons of sympathetic preganglionic neurons. Recordings of population responses in individual ganglia with suction electrodes attached to the inter-ganglion root were undertaken to characterize the plasticity in sympathetic chain ganglia after spinal cord injury. While preliminary and very early after SCI, sympathetic chain ganglia from both these animals underwent spontaneous bursts of activity that initiated long- lasting membrane oscillations. When recording from the T12 ganglia, in animals that had undergone T2 spinalization previously, we observed that spontaneous activity could initiate or abolish ongoing persistent oscillatory activity that was long-lasting (seconds to over 30 minutes). Ventral root stimulation could also abolish ongoing oscillatory behavior. Oscillation frequencies were between ~10-20Hz. Strikingly, bursts acted as switches between oscillatory and quiescent states, suggestive of a SPN circuit-based capacity for the induction of membrane bistability. In comparison, only 1 of 25 control animals exhibited this behavior, and only in the presence of atropine. Furthermore, in the animal that had undergone SCI 5 days prior, ventral root stimulation evoked long- lasting bouts of spontaneous activity that was abolished following nicotinic receptor block with mecamylamine, supporting activity induced by preganglionic nicotinic actions on postganglionic neurons. Studying these neuroplastic changes in sympathetic postganglionic neurons following SCI will allow us to understand how synaptic alterations contribute to autonomic dysfunction. Further studies are proposed. Supported by the Department of Defense (Awards #25530)

2. **M McKinnon, M Choi, S Hochman**. Characterization of cellular and synaptic properties in adult mouse thoracic paravertebral ganglia. *Pre-meeting on rhythmic motor circuits. 10/16/2015.*

Thoracic intraspinal **pre**ganglionic neurons constitute the entire CNS sympathetic output. They project to sympathetic **post**ganglionic neurons within autonomic ganglia. A major function of sympathetic paravertebral chain ganglia neurons is to maintain vasomotor tone. While the functional properties of cervical and lumbosacral paravertebral ganglia neurons have been characterized, little is known about the functional properties of neurons within thoracic paravertebral ganglia. We developed an approach that allows for whole-cell patch clamp recordings in intact thoracic ganglia to characterize cellular and synaptic properties.
We recorded from 5 cells, 2 of which were of adequate quality to characterize passive membrane properties. Preliminary findings from whole-cell recordings (n=2) in fifth right thoracic ganglia show that cells have input resistance around 485MΩ, resting membrane potential -55 to -50 mV, and membrane time constant of 85 ms. Threshold voltage was typically 10 mV higher than resting membrane potential, action potentials displayed after-hyperpolarization and some cells displayed post-inhibitory rebound. Maximal firing rates observed in response to depolarizing current steps ranged from 14-17 spikes/sec. During intracellular depolarization, firing rate increases with increased current injection and cells sustain tonic firing.
Preliminary examination of nicotinic acetylcholine receptor (nAChR) activation was studied in one neuron from the fifth right thoracic ganglion. When perfused with 100uM acetylcholine, this cell

depolarized from -60mV to -40mV and was observed to fire at a maximal instantaneous firing rate of 15 Hz. Spike frequency adaptation was also observed; spiking gradually stopped as membrane potential repolarized to -30mV around 40s after initiation, presumably by receptor desensitization. Choline also led to a modest membrane depolarization. In both cases, depolarizing response was attenuated in the presence of nAChR antagonist, hexamethonium.

Patch clamp recordings in combination with optogenetics in another cell from the seventh right thoracic ganglion from a ChAT-ChR2 mouse suggested that postganglionic neurons receive massive cholinergic input from preganglionic neurons. Repetitive (10 Hz) optical stimulation of cholinergic preganglionic axons elicited a large EPSP in the postganglionic neuron (20 mV). The EPSP was usually suprathreshold and accompanied by an action potential. The response fatigued dramatically to repetitive stimuli. This seems to indicate that presynaptic release of acetylcholine attenuates rapidly on the order of 100-300 milliseconds. Together, these data suggest that presynaptic release of acetylcholine by preganglionic neurons is the rate-limiting step for transmission in thoracic paravertebral ganglia.

By using a ChAT-cre x LacZ reporter mouse which labels a sparse subpopulation of cholinergic preganglionics, we were able to observe individual synaptic arborizations. Each arborization within thoracic paravertebral ganglia was observed to surround a single postganglionic neuron with several (10-20) large axosomatic synapses. This organizational principle, a one-to-one connection between pre- and postganglionic neurons, is in line with observations from other sympathetic ganglia.

EARLY INSIGHT INTO THE EMERGENCE OF SPONTANEOUS SYNCHRONOUS OSCILLATORY ACTIVITY IN ISOLATED THORACIC CHAIN GANGLIA AFTER SCI

Mallika Halder, Michael McKinnon, Michael Sawchuk, Shawn Hochman

Physiology Department at Emory University School of Medicine, Atlanta, Georgia USA.

INTRODUCTION

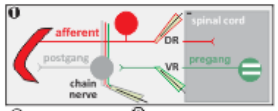
Paravertebral sympathetic ganglia control vasomotor function in the upper and middle extremities. Loss of descending control of their function as occurs after spinal cord injury (SCI) is associated with the emergence of dysautonomias. Particularly severe cardiovascular dysfunction, including autonomic dysreflexia, occurs in individuals with lesions above T6 that compromise brainstem control over the heart and splanchnic vasculature. Both are important in the effective regulation of blood pressure. It is thus no surprise that cardiovascular disease is the most common cause of death after SCI.

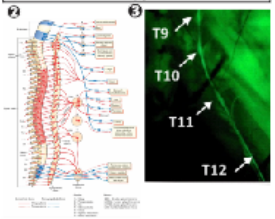
We speculate that observed dysfunction occurs in part via changes within sympathetic postganglionic neurons (SPNs) located in more caudal thoracic paravertebral sympathetic chains (tSPNs).
More caudal tSPNs are practically inaccessible for *in vivo* studies and there are only a few studies *in vitro* on caudal tSPNs in guinea pig[1,2].
We developed an *in vitro* adult mouse model that retains thoracic sympathetic chain ganglia *in situ*. It has attached ventral roots for stimulation of preganglionic axons as well as intact dorsal root ganglia and dorsal roots.

Below we use predominantly extracellular recordings to provide the first insights into plasticity in tSPNs after SCI.

in vitro Mouse Model

- The thoracolumbar vertebral column of of ChAT-eGFP mice (P60 or older) was isolated and placed in a recording chamber perfused with artificial cerebrospinal fluid.
- The spinal cord was removed following a dorsal laminectomy and ventral vertebrectomy to provide access to dorsal and ventral roots.
- Stimulating suction glass electrodes were placed on ventral roots to recruit axons of sympathetic preganglionics. Electrodes were also placed on dorsal roots to examine primary afferent evoked responses.
- A recording suction electrode was placed on the interganglionic nerve root against caudal thoracic ganglia (with a focus on T12) to assess population postganglionic responses
- Comparisons were made between controls and mice spinalized at T2 at 1 day, 5 days and 6 days post injury.

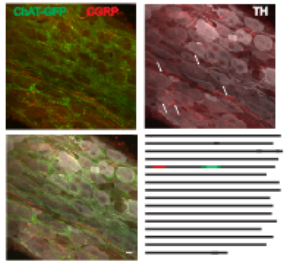




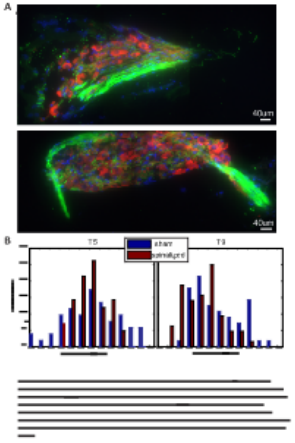
RESULTS

CHAIN ANATOMY

1. PREGANGLIONIC, POSTGANGLIONIC AND CGRP+ AFFERENT AXONS ARE FOUND IN SPN GANGLIA.

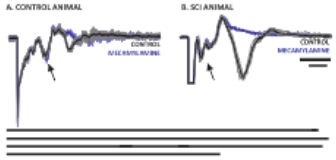


2. PRELIMINARY CHARACTERIZATIONS SUGGESTS POSSIBLE CHANGE IN CELL DIAMETERS & POPULATION TYPES AFTER SCI

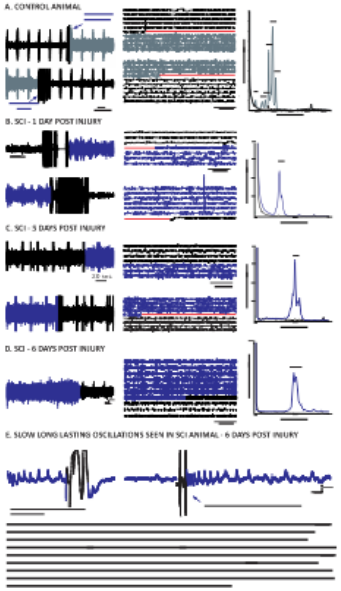


FEATURES OBSERVED AFTER SCI

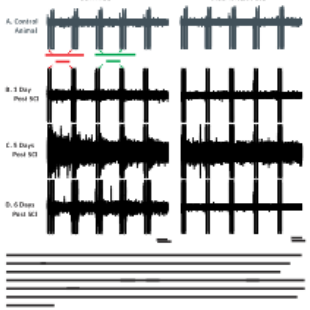
1. VENTRAL ROOT EVOKED RESPONSES ARE nAChR MEDIATED



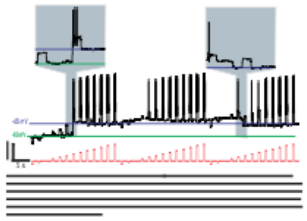
2. LONG-LASTING OSCILLATIONS PREFERENTIALLY EMERGE IN SCI MICE



3. INCREASED EXCITABILITY FOLLOWING VENTRAL ROOT STIMULATION.



4. BISTABLE STATE CHANGE IN ISOLATED CHAIN GANGLIA: POSSIBLE EXPLANATION FOR SPONTANEOUS OSCILLATIONS POST SCI.



SUMMARY OF SPINALIZED ANIMALS TESTED IN THESE STUDIES

DISCUSSION

We developed an *in vitro* mouse model that allows access to the paravertebral sympathetic chain for electrophysiological studies.

Pre-ganglionic, post-ganglionic and CGRP+ afferent axons were observed in interganglionic nerves between sympathetic chain ganglia suggesting considerable intersegmental interactions.

Preliminary differences in cell sizes and populations in thoracolumbar SPN ganglia before and after SCI shows that the range of TH+ neurons reduces after spinal cord injury in the T5 ganglia. Cell diameters of spinalized animals are smaller than sham animals.

Ventral root evoked responses in T12 sympathetic chain ganglia are partially nAChR mediated and can be blocked with nAChR antagonists.

Long lasting spontaneous oscillations induced by spontaneous events, mechanical and high intensity 10 Hz stimulation were present in 3/4 spinalized animals tested compared to the 1/27 control animals. Frequency of oscillatory behavior ranged from 10-17Hz and could be switched on or off with spontaneous events.

A slower long lasting oscillation induced by 10Hz stimulation and spontaneous activity was also observed in an animal 6 days after injury.

10Hz ventral root (but not dorsal root) stimulation increases excitability via nAChR mechanisms 5 and 6 days after injury. Increased spiking can be blocked with nAChR antagonist mecamylamine. No control animals showed an increase in excitability following 10Hz stimulation.

Intracellular recordings from a T5 ganglia cell shows evidence of a bistable state change. The possibility that a group of ganglia cells can shift to a high state and can synchronize to fire at a coherent frequency is consistent with the membrane property of bistability and offers an explanation for themechanism involved in spontaneous evoked oscillations seen after SCI. This is important for the way one might think about autonomic dysreflexia. The model has no spinal cord or brain stem but can shift into a high output states.

REFERENCES

1 Blackman, J. G. & Purves, R. D. Intracellular recordings from ganglia of the thoracic sympathetic chain of the guinea-pig. J Physiol. 203, 173-198. (1969).

2 Lichman, J. W., Purves, D. & Yip, J. W. Innervation of sympathetic neurones in the guinea-pig thoracic chain. J Physiol. 298, 285-299. (1980)

3 Jobling, P. & Gibbins, I. L. Electrophysiological and morphological diversity of mouse sympathetic neurons. Journal of neurophysiology 82, 2747-2764 (1999).

ACKNOWLEDGEMENTS

Supported by
Department of Defense SCI-30225

CHARACTERIZATION OF CELLULAR AND SYNAPTIC PROPERTIES IN ADULT MOUSE THORACIC PARAVERTEBRAL GANGLIA

M.L.McKinnon, M. Sawchuk, C. J. MacDowell, M. H. Choi, S. Hochman
Dept. Physiol, Emory Univ Sch Med, Decatur, GA

EMORY UNIVERSITY

BACKGROUND

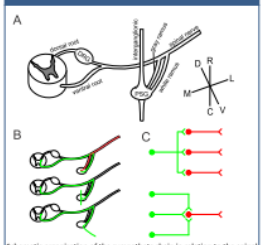
The sympathetic chain comprises a group of interconnected sympathetic ganglia situated in the ventral side of the vertebral column. Whereas other sympathetic ganglia are typically associated with one or more visceral organs in a discrete location (coeliac ganglion, superior/inferior mesenteric ganglion), chain ganglia are associated with distributed organ systems such as vasculature, sweat glands, and piloerector muscles. As such, the sympathetic chain can be thought of as a distribution system for sympathetic activity that must span the body.

Almost nothing is known of mammalian paravertebral thoracic sympathetic chain ganglia. The electrical properties of sympathetic neurons have been studied in the superior cervical ganglion and to a lesser extent the stellate and lumbar ganglia, but to date only 3 studies have revealed electrophysiological properties of thoracic ganglia 1-3. Yet thoracic chain ganglia prominently control vasomotor actions as they leave their corresponding spinal nerves to regulate vasculature in upper and middle extremities as well as in bronchi, lungs, heart, esophagus, integumentary system and some viscera[4].

There are few studies on most thoracic paravertebral sympathetic ganglia because they are physically inaccessible for in vivo study as they are deeply embedded and hidden within adherent fat attached adjacent to the ventral vertebral column. Thus, studies have focused on the much more accessible cervical [5-7] and lumbar ganglia [8,9]. Paravertebral ganglia are thought to receive highly convergent input from preganglionics. For example, superior cervical ganglion preganglionic axons arise from many spinal segments and innervate between 50 and 240 SPNs [10,11]. A similar relationship may hold true for thoracic ganglia 2, but may not. For example, the firing of postganglionics in lumbar ganglia may be driven by 2 to 3 preganglionic neurons [8].

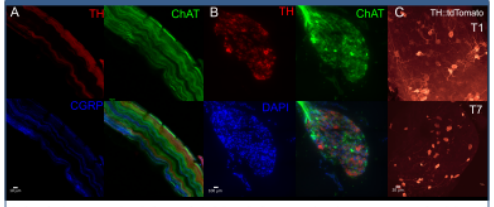
Below, we report our initial work on the integrative properties of thoracic chain postganglionic neurons. This includes: (i) anatomical properties, (ii) patterns of convergence onto individual ganglia and multisegmental divergence onto many chain ganglia, (iii) postganglionic neuronal firing properties, and (iv) synaptic properties.

ANATOMICAL SCHEMATIC



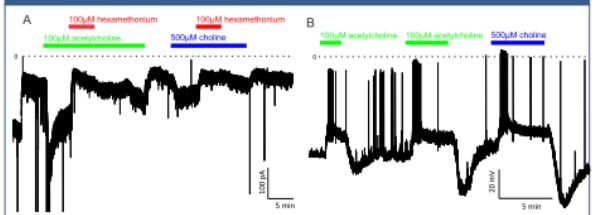
Schematic organization of the sympathetic chain in relation to the spinal cord. A: The paravertebral sympathetic chain ganglia lie ventral to the spinal column and are enmeshed in fat. Ganglia are connected to the spinal nerve via white and gray rami communicans, and to eachother through the interganglionic nerve. **B:** Sympathetic preganglionic neurons in the thoracolumbar spinal cord project throughthe ventral root and enter the sympathetic chain via the white ramus communicans.Axons may either synapse onto a postganglionic neuron, travel up or down the chain any number of segments, or exit the chain to innervate prevertebral ganglia (superior/inferior mesenteric, celiac). **C:** A single preganglionic neuron may diverge and innervate several postganglionic neurons in different segments. Likewise, several preganglionic neurons from different spinal segments may innervate the same postganglionic.

HISTOLOGY



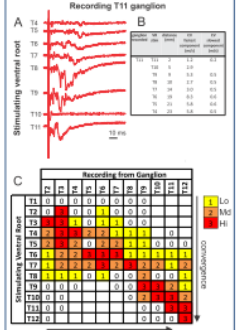
Histology of major neurotransmitters in the sympathetic chain. A: The thoracic sympathetic chain comprises a group of regularly spaced ganglia of various sizes, interconnected by an interganglionic nerve. Axons within interganglionic nerves are TH+ postganglionic adrenergic, ChAT+ preganglionic, and CGRP+ visceral afferents. **B:** Most cells are adrenergic and identified by immunolabeling for tyrosine hydroxylase (TH), while a minority (3-5%) are cholinergic, identified by their expression of choline acetyltransferase (ChAT). **C:** Cells somata are variable in size with diameters ranging from 10-35um. Rostral thoracic neurons have dendrites while caudal thoracic neurons lack dendrites.

RECEPTOR PHARMACOLOGY



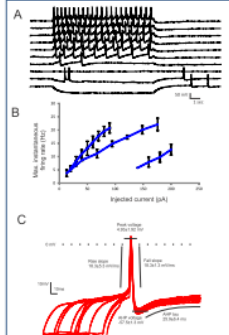
Cells respond to nAChR agonists and antagonists. As expected, postganglionic neurons are excited by ACh via activation of nAChRs that are sensitive to block via hexamethonium. Interestingly, nAChR-mediated excitatory responses were also seen following application of choline, an a7 nAChR receptor agonist. Responses are shown in voltage clamp (**A**) and current clamp (**B**).

SPINAL CORD INPUT



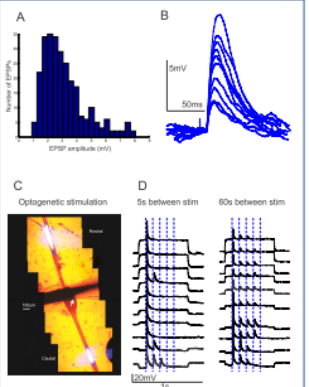
Sympathetic chain ganglia exhibit convergence and divergence of inputs. A: Example of convergent input onto the T13 ganglion. **B:** Conduction velocities range between 0.2-8.3 m/s - comparable to that observed in guinea pig by Blackman & Purves (1969). **C:** Map of convergence and divergence in another animal. Estimates of preganglionic convergence onto individual ganglia are shown as columns progressing from T2-T12 color-coded to projection magnitude. Preganglionic divergence is shown in rows.Properties of convergence and divergence are comparable to those seen in guinea pig [Lichtman et al 1980].

FIRING PROPERTIES



Firing properties of paravertebral neurons. A recent study has shown that sharp microelectrode recordings do not reliably reflect passive membrane or firing properties (Springer et al 2015). We have begun the first whole-cell patch clamp recordings in thoracic paravertebral ganglia. **A:** Thoracic ganglion neurons fire tonically in response to sustained depolarizing current, and exhibit rebound spiking. This was also seen in superior cervical ganglia by Springer et al (2015). **B:** Firing rate increases linearly in response to increases in depolarizing current, but rheobase and response properties are variable between cells. **C:** Action potentials have a rising and falling slope of 18.3 mV/ms and exhibit afterhyperpolarization with a decay tau of 25.9 ms.

SYNAPTIC PROPERTIES



Synaptic properties of paravertebral neurons. A: Spontaneous EPSP amplitudes occupy a continuous range from 1 to 8mV. **B:** Overlaid traces of representative EPSPs. **C:** We use optogenetics to investigate synaptic properties of cells. Using ChAT-cre::ChR2 mice, we are able to selectively stimulate preganglionic axons in the interganglionic nerve. **D:** Activation of preganglionic axons leads to a massive (20-40 mV) EPSP in the postganglionic cell. This response fatigues rapidly with repetitive 10Hz stimulation.

DISCUSSION

- Sympathetic chain ganglia represent an understudied neuronal population due to the difficulty in accessing these ganglia
- Previous studies which sought to characterize electrophysiological properties in these ganglia were flawed due to their recording technique.
- By utilizing patch clamp recordings rather than sharp microelectrode recordings, the passive and firing properties of thoracic sympathetic neurons are preserved.
- Optogenetic studies have furthermore allowed us to selectively target cholinergic preganglionic axons to study synaptic properties of thoracic sympathetic neurons.

REFERENCES

1.Blackman, J. G. & Purves, R. D. Intracellular recordings from ganglia of the thoracic sympathetic chain of the guinea-pig. J Physiol. 203, 173-198. (1969).
2.Lichtman, J. W., Purves, D. & Yip, J. W. Innervation of sympathetic neurones in the guinea-pig thoracic chain. J Physiol. 286, 185-200. (1980).
3.Jobling, P. & Gibbins, I. L. Electrophysiological and morphological diversity of mouse sympathetic neurons. Journal of neurophysiology 82, 2787-2794 (1999).
4.Janig, W. The Integrative Action of the Autonomic Nervous System. (Cambridge University Press, 2006).
5.Campanucci, V., Krishnaswamy, A. & Cooper, E. Diabetes depresses synaptic transmission in sympathetic ganglia by inactivating nAChRs through a conserved intracellular cysteine residue. Neuron. 66, 827-834. doi: 810.1016/j.neuron.2010.1006.1030. (2010).
6.Nirenberg, K. & Hoon, J. P. Weak and straddling secondary nicotinic synapses can drive firing in rat sympathetic neurons and thereby contribute to ganglionic amplification. Frontiers in neurology 1, 138. doi:10.3389/fneur.2010.00138 (2010).
7.Springer, M. G., Rollmann, P. H. & Hore, J. P. Virtual leak channels modulate firing dynamics and synaptic integration in rat sympathetic neurons: implications for ganglionic transmission in vivo. The Journal of physiology 593, 803-823. doi:10.1113/jphysiol.2014.284125 (2015).
8.Bratton, B., Davies, P., Janig, W. & McAllen, R. Ganglionic transmission in a vasomotor pathway studied in vivo. J Physiol. 586, 1647-1664. doi: 1610.1113/jphysiol.2008.165920. Epub 162008 Mar 165920. (2010).
9.Perry, W. H. & Knox, J. Cholinergic excitatory synaptic potentials of neurones in mammalian lumbar paravertebral ganglia. Journal of the autonomic nervous system 18, 195-205 (1987).
10.North, R. A. Mechanisms of Autonomic Integration. Handbook of Physiology, The Nervous System, Intrinsic Regulatory Systems of the Brain, 115-153 (1986).
11.Wang, F. B., Holst, M. C. & Powley, T. L. The ratio of pre- to postganglionic neurons and related issues in the autonomic nervous system. Brain research. Brain research reviews 25, 93-115 (1998).

Supported by Department of Defense SCI-30225